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ORIGINAL PAPER

Influence of cytokinins, basal media and pH on adventitious shoot regeneration from excised root cultures of *Albizia lebbeck*

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Abstract: A highly reproducible and efficient *in vitro* shoot regeneration system was developed in a potential medicinal plant, *Albizia lebbeck* using root explants. Root explants from 15 day-old-aseptic seedlings were cultured on Murashige and Skoog (MS) medium supplemented with different concentrations (0.5, 2.5, 5.0, 7.5 and 10.0 µM) of 6-Benzyladenine (BA), Kinetin (Kn), 2-Isopentenyl adenine (2-iP) singly as well as in combination with α-Naphthalene acetic acid (NAA) (0.1, 0.5, 1.0, 1.5 and 2.0 µM). The highest rate of shoot multiplication (16.0 ± 1.87 for the average shoot number and 5.16 ± 0.38 cm for shoot length) was achieved on MS medium supplemented with 7.5 µM BA and 0.5 µM NAA. The effects of medium type, medium strength, pH and subculture on shoot induction and proliferation were also tested. An average of 21.6±2.87 shoots per explants could be obtained following this protocol. Rooting was achieved on microshoots using half strength MS medium with 2.0 µM Indole-3-butyric acid (IBA) after four weeks of culture. The *in vitro* raised healthy plantlets were successfully established in earthen pots containing garden soil and grown in greenhouse with >80% survival rate.

Keywords: *Albizia lebbeck*; direct organogenesis; fabaceae; plant regeneration; root explants

Introduction

Albizia lebbeck (L.) Benth., commonly known as Shirish or Woman's Tongue tree, belonging to the family Fabaceae, is an erect, deciduous, mimosoid legume. It is distributed throughout India, Bangladesh, tropical and subtropical regions of Asia and Africa. Its bark is used for toothache and diseases of the gum. Seeds are astringent and are given in piles and diarrhoea. Decoction of the leaves and barks are protective against bronchial asthma and other allergic disorders (Saha and Ahmad 2009). The methanolic extract of the pod was investigated for antifertility activity (Gupta et al. 2005). The plant extract was also evaluated in allergic rhinitis (Pratibha et al. 2004) and memory and learning of mice (Chintawer et al. 2002). The plant parts are rich in macrocyclic alkaloids (Dixit and Mishra 1997), phenolic glycosides (Maa et al. 1997), flavonoids (El-Mousallamy 1998) and saponins (Ueda et al. 2003). In addition, antiprotozoal, hypoglycemic, anticancer and analgesic properties (Saha and Ahmad 2009) have also been reported in this plant.

Indiscriminate and illegal logging, low natural regenerative potential, narrow habitat specificity, microclimatic changes and long seed dormancy have resulted in the severe depletion of natural population of *A. lebbeck* (Troup 1986). Seeds have been the most widely used means for propagating the prolific *A. lebbeck*, although vegetative propagation via stem cuttings can be useful for improving the genetic quality of the planting stock. However, conventional propagation is not brisk to meet the needs in time. On the other hand, biotechnological approaches can be employed for plant improvement through somaclonal variation and genetic transformation as well as for the commercial exploitation of valuable plant-derived pharmaceuticals (Chaudhuri et al. 2004). Therefore, tissue culture techniques provide viable alternative methods for the mass production of healthy plants with uniform characteristics.

Earlier reports on *A. lebbeck* describe the regeneration of plants through hypocotyl, root, cotyledon, leaflet (Gharyal and Maheshwari 1983; Mamun et al. 2004), stem, petiole (Gharyal and Maheshwari 1990) and nodal explants (Mamun et al. 2004) using direct and indirect regeneration methods. There is only one report on the regeneration from root explant (Gharyal and Maheshwari 1983) which yielded low number of shoots and cannot be used for large scale multiplication. Therefore, the ob-

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jective of this study was to develop an efficient and improved method for rapid *in vitro* propagation of *A. lebbeck* using root explants via optimization of basal media, pH level, cytokinins and auxin followed by successful field establishment of regenerated plants.

Materials and methods

Seed germination and establishment of aseptic seedling

The mature fruits of *A. lebbeck* were collected from a 25 year-old tree growing in Botanical Garden of the University, Aligarh. The healthy seeds were excised from the fruits and washed thoroughly in running tap water for 30 min. These were immersed in 1% (w/v) solution of Bavistin, a fungicide, for 15 min to remove adherent particles from the surface and later treated with 5% (v/v) Teepol solution for 20 min. Thereafter, the seeds were rinsed thrice with sterile distilled water followed by surface sterilization in 0.1% (w/v) $HgCl_2$ solution under laminar flow hood for 15 min. Finally, the seeds were rinsed thrice with sterile distilled water to remove the traces of $HgCl_2$ solution. The disinfected seeds were inoculated on MS (Murashige and Skoog 1962) basal medium for seed germination. Root segments excised from the sub- apical (position immediately after the apical white root segment) to basal region of roots of 15-day-old seedlings were used as explants.

Culture media and culture condition

MS (Murashige and Skoog 1962) basal medium fortified with 3% (w/v) sucrose, 0.8% (w/v) agar (Qualigens, Mumbai, India) was used in all the experiments. The medium was adjusted to different pH levels viz 5.0, 5.4, 5.8, 6.2 and 6.6 using 1N NaOH or HCl and sterilized by autoclaving at 121°C and 1.06 $Kg \cdot cm^{-2}$ pressure for 20 min. Cultures were incubated at 25±2°C under 16/8 h (light/dark) photoperiod provided by cool white fluorescent tubes (Phillips, India) with a photon flux density of 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at 55%–60% relative humidity.

Adventitious shoot induction and multiplication

Root explants excised from 15-day-old seedlings were cultured onto MS medium augmented with various concentrations of 6-Benzyladenine (BA), Kinetin (Kn), and 2-Isopentenyl adenine (2-iP) (0.5, 2.5, 5.0, 7.5 and 10.0 µM) singly as well as in combination with different concentrations of α-Naphthalene acetic acid (NAA) (0.1, 0.5, 1.0, 1.5 and 2.0 µM) for induction and multiplication of shoots. Different basal media namely, Murashige and Skoog medium (MS), ½ MS, Woody Plant Medium (WPM) (Lloyd and McCown 1980), ½ WPM, Gamborg's Medium (B_5) (Gamborg et al. 1968), ½ B_5 and pH level including 5.0, 5.4, 5.8, 6.2 and 6.6 were tested to identify the most suitable medium and pH for shoot multiplication. Each basal medium and pH was supplemented with optimal combination of growth regulators. Subculturing was performed on the same fresh media at every two weeks to avoid basal callusing. Data on the frequency of explants producing shoots, shoot number and shoot length were recorded after four weeks of culture and the shoot forming capacity (SFC) index was calculated according to Martinez-pulido et al. (1992) as follows; SFC index = % explants with shoots × Mean no. of shoots per explants /100

In vitro rooting of shoots and transfer of plantlets to soil

Shoots with fully expanded leaves were excised from the root explants and transferred to 1/2 strength MS Medium supplemented with either Indole-3-butyric acid (IBA) or NAA at various concentrations including 0.5, 1.0, 1.5, 2.0 and 2.5 µM. Observations on percent rooting, number of roots per shoot and root length were recorded after four weeks.

Shoots with well developed roots were removed from the culture vessels and rinsed with running tap water to remove agar. Plantlets were transferred to plastic cups containing soilrite and incubated under diffuse light (16/8 h photoperiod) conditions. Potted plantlets were covered with transparent plastic bags to ensure high humidity and watered at regular intervals with ½ strength MS salt solution for three weeks. Thereafter, bags were removed to acclimatize the plantlets to field conditions. After four weeks, acclimatized plants were transferred to pots containing garden soil and maintained in greenhouse under normal day length conditions.

Statistical analysis

Each treatment consisted of 10 replicates and all experiments were repeated thrice. The data were analyzed statistically using One Way Analysis of Variance (ANOVA) and pairwise means were compared using Duncan's multiple range test procedure (p = 0.05) (Duncan 1955).

Results and discussion

Effect of plant growth regulators on direct shoot induction and multiplication

The morphoregulatory potential of various plant growth regulators on root explants was explored and summarized in Table 1 and Table 2. Culture medium devoid of plant growth regulators failed to stimulate adventitious shoot bud differentiation from root explants even though the explants are cultured for more than four weeks. The explants in the control remained fresh for about three weeks but thereafter turned brown and died. In contrast, all these tests revealed that there were significant differences (p=0.05) in shoot induction and multiplication with the variation of BA, Kn, 2-iP singly or in combination with NAA tested (Table 1 and Table 2) concentrations of BA, Kn and 2-iP facilitated adventitious shoot bud regeneration after four weeks of culture. Root segments showed swelling and developed greenish patches after two weeks of culture. These green color regions differentiated into shoot buds after four weeks (Fig 1a).

Table1. Effect of various cytokinins (BA, Kn, 2iP) on multiple shoot bud induction from root explants of *A. lebbeck* after four weeks of culture

Plant growth regulators (µM) BA	Kn	2-iP	% response	Mean no. of shoot buds/ root explants	SFC index (%)
0.5			58.6 ± 0.88bcd	3.20 ± 0.66^{def}	1.87
2.5			63.3 ± 2.60abc	5.20 ± 0.86^{cd}	3.29
5.0			66.3 ± 2.90ab	7.20 ± 1.01^{a}	4.77
7.5			74.6 ± 1.70a	10.0 ± 2.62^{a}	7.46
10			70.3 ± 1.82ab	7.40 ± 1.56^{a}	5.2
	0.5		38.3 ± 4.10ef	1.80 ± 0.66^{ef}	0.68
	2.5		48.6 ± 5.40cde	2.80 ± 0.73^{def}	1.36
	5.0		50.6 ± 5.80cde	4.80 ± 0.96^{cde}	2.42
	7.5		57.6 ± 4.90^{bcd}	6.60 ± 0.92^{c}	3.8
	10		55.0 ± 3.60bcd	4.80 ± 0.58^{cde}	2.64
		0.5	32.0 ± 2.30f	0.90 ± 0.45^{f}	0.28
		2.5	35.6 ± 8.25ef	2.20 ± 0.73^{def}	0.78
		5.0	44.0 ± 7.76def	3.20 ± 0.86^{def}	1.40
		7.5	49.6 ± 5.40cde	4.60 ±0.67^{cde}	2.28
		10	44.3 ± 5.70^{def}	2.80 ±0.58^{def}	1.24

Values represent means ± SE. Means sharing the same letter within columns are not significantly different (p = 0.05) using Duncan's multiple range test.

Table 2. Effect of various concentrations of NAA with optimal concentration of BA, Kn, and 2- iP on shoot multiplication and elongation of *A. lebbeck* after four weeks of culture

Plant growth regulators (µM) BA	Kn	2-iP	NAA	% response	Mean no. of shoot buds/ root explants	Mean Shoot length (cm)
7.5			0.1	79.3 ± 0.88^{ab}	13.0 ± 3.00^{ab}	4.20 ± 0.46^{ab}
7.5			0.5	84.0 ± 1.73^{a}	16.0 ± 1.87^{a}	5.16 ± 0.38^{a}
7.5			1.0	75.6 ± 2.18^{ab}	12.2 ± 2.50^{ab}	3.70 ± 0.43^{bc}
7.5			1.5	67.3 ± 2.60^{bcd}	11.2 ± 2.43^{abc}	4.10 ± 0.48^{ab}
7.5			2.0	69.0 ± 2.30^{bc}	10.4 ± 2.42^{abcd}	3.96 ± 0.47^{ab}
	7.5		0.1	54.0 ± 2.88^{ef}	7.60 ± 1.93^{bcdef}	2.06 ± 0.19^{de}
	7.5		0.5	71.0 ± 1.52^{bc}	9.40 ± 0.60^{bcde}	3.04 ± 0.32^{bcd}
	7.5		1.0	68.0 ± 1.15^{bcd}	7.20 ± 1.74^{bcdef}	2.40 ± 0.40^{cde}
	7.5		1.5	56.0 ± 3.05^{def}	4.40 ± 1.69^{defg}	2.50 ± 0.68^{cde}
	7.5		2.0	52.6 ± 4.63^{ef}	3.20 ± 1.93^{f}	2.90 ± 0.45^{bcde}
		7.5	0.1	53.3 ± 3.17^{ef}	2.20 ± 0.86^{f}	1.48 ± 0.18^{e}
		7.5	0.5	61.6 ± 2.02^{cde}	6.00 ± 1.00^{cdef}	2.80 ± 0.66^{bcde}
		7.5	1.0	48.3 ± 7.26^{f}	4.00 ± 2.44^{ef}	1.40 ± 0.42^{e}
		7.5	1.5	50.0 ± 7.0^{ef}	2.40 ± 1.12^{f}	2.40 ± 0.50^{cde}
		7.5	2.0	49.0 ± 6.65^{ef}	1.40 ± 0.97^{f}	1.50 ± 0.44^{e}

Values represent means ± SE. Means sharing the same letter within columns are not significantly different (p = 0.05) using Duncan's multiple range test.

The data were analyzed statistically using ANOVA (variance analysis) and Duncan's test. The parameters evaluated consisted of percent of regenerated root explants (number of root explants inducing shoots after four weeks and eight weeks / total number of explants), mean number of shoots (number of regenerated shoots/explants), shoot length and percent of rooting shoots (number of root-producing shoots/number of regenerated shoot), number of roots/ shoot and root length.

Fig. 1 (a-e) *In vitro* plant regeneration of *Albizia lebbeck* L. Benth. (a) Multiple shoot induction in root explant on MS medium supplemented with BA (7.5 µM) after four weeks of culture. (b&c) Shoot multiplication and proliferation on MS medium augmented with (7.5 µM) BA and (0.5 µM) NAA after eight weeks of culture. (d) *In vitro* rooting in microshoot on half strength MS medium supplemented with IBA (2.0 µM) after four weeks of culture. (e) An acclimatized plantlet in soilrite.

MS medium containing 7.5 µM BA induced greater percentage of shoot organogenesis (74.6%) as compared to other cytokinins used in this study. In addition, a significantly greater number of shoot buds were observed on this medium (Fig. 1b). At the same concentration, Kn and 2-iP produced 6.60 ± 0.92 and 4.60 ± 0.67 shoot buds in 57.6% and 49.6% cultures, respectively. MS medium supplemented with BA gave a better response than Kn and 2-iP but the influence of BA (7.5 µM) was statistically not significant (p = 0.05) to Kn (7.5 µM) and 2-iP (7.5 µM). Ovecka et al. (2000) reported that cell competence in the course of shoot bud regeneration is controlled by various internal factors such as genotype, endogenous level of auxin and cytokinin, basal medium, pH, carbohydrate uptake, etc. The promoting effect of BA on bud regeneration from root explants has been reported in other woody plant species such as *Citrus mitis* (Sim et al. 1989), *Populus tremula* (Vincour et al. 1997), *Citrus aurantifolia* (Bhat et al. 1992), *Melia azedrach* (Vila et al. 2005) and *Aralia elata* (Karim et al. 2007). On increasing the level of BA from 7.5 to 10 µM, the percent regeneration as well as number of shoots was drastically reduced, which resulted in the formation of diminutive and fasciated shoots. Similar observation is substantive with the report documented by Husain and Anis (2004) in *Melia azedrach*, where BA at 10 µM showed a decrease in the rate of shoot multiplication. Such an inhibitory effect at higher concentration of BA has also been reported by other workers irrespective of explants used (Chaudhuri et al. 2004; Ahmad et al. 2008). For further enhancement in multiplication of shoots, the optimal concentration of BA, Kn and 2-iP (7.5 µM) in combination with NAA at different concentrations

(0.1–2 µM) was also assayed. The inclusion of NAA in the medium enhanced the shoot multiplication rate in *A. lebbeck*. Kim et al. (2001) suggested that the shoot forming ability of the explants is related to the *in vivo* level of endogenous auxin and cytokinin and that the differential response to different cytokinins may be because of the chemical and structural differences. Among the various cytokinins- auxin combination used, the combined effect of BA (7.5 µM) and NAA (0.5 µM) was significant for shoot multiplication where the highest number of shoots (16.0±1.87) and shoot length (5.16±0.38 cm) were recorded after eight weeks of culture (Table 2) (Fig. 1c). In this study, Kn (7.5 µM) and NAA (0.5 µM), and 2-iP (7.5 µM) and NAA (0.5 µM) were less effective than BA (7.5 µM) and NAA (0.5 µM) but significantly (p = 0.05) better than other treatments.

It is reported that a good combination of cytokinins and auxin in the culture medium enhanced good shoot formation and plantlet regeneration from explants, e.g., Mamun et al. (2004) obtained direct shoot bud regeneration from different explants (*in vitro* cotyledons, nodal segments and *in vivo* nodal segments) of *A. lebbeck* on MS medium supplemented with BA and NAA. About 7.3 shoots were produced from *in vitro* nodal segments on MS medium containing BA (2.5 mg/L) and NAA (0.2 mg/L) after six weeks of incubation. On contrary, in this study, about 16.0±1.87 shoots were induced on MS medium containing BA (7.5 µM) and NAA (0.5 µM) from excised root segment after eight weeks of culture. Our results are in agreement with the findings of Kantharajah et al. (1992) in *Averrhoa carambola*, Georges et al. (1993) in *Lonicera japonica* and Shahin-uz-zaman et al. (2008) in *Azadirachta indica*, where BA and NAA combination was the best in inducing direct caulogenesis from root culture.

The shoot cultures were multiplied by repeated subculturing of regenerative tissue on MS medium with BA (7.5 µM) and NAA (0.5 µM). The subculturing of organogenic tissue enhanced shoot development (21.6±2.87) until 4th subculture passage. However, extending the subculture period above this period caused a decline in multiplication rate (Fig. 2). Similar effect of subculturing on shoot multiplication was also reported by Hiregoudar et al. (2006) in *Vitex trifolia* and Siddique and Anis (2009) in *Balanites aegyptiaca*.

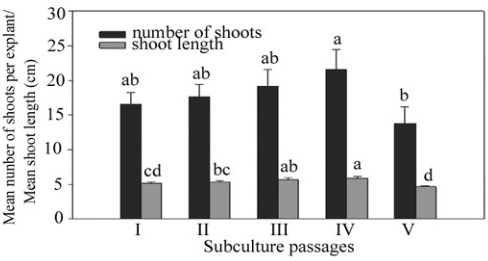

Fig. 2 Effect of subculture passages on shoot multiplication and elongation from root explants of *A. lebbeck* in MS medium supplemented with BA (7.5 µM) + NAA (0.5 µM) after five subculture passages. Bars represent the means ± SE. Bars denoted by the same letter within response variables are not significantly different (*p*= 0.05) using Duncan's multiple range tests.

Effect of different basal media on shoot regeneration

The highest rate of micropropagation will often depend not only on the selection of the most suitable explants, but also on the best basal medium for that tissue (Basu and Chand 1996; Martin 2004). The nutritional requirement varies according to the cells, tissues, organ and protoplast and also with respect to particular plant species. In the present investigation three basal media i.e., MS, WPM and B_5, were tested for their influences on adventitious shoot regeneration and multiplication. All three basal medium were tried in full and half strength (MS, ½MS, WPM, ½WPM, B_5, ½B_5), in which full strength medium supplemented with optimal concentration of plant growth regulators proved best as compared to half strength media (Fig. 3).

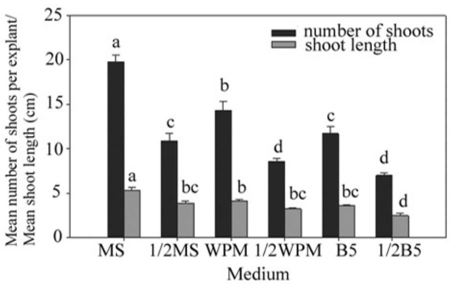

Fig. 3 Effect of different basal medium on shoot regeneration from root explants of *A. lebbeck* supplemented with BA (7.5 µM) + NAA (0.5 µM) after eight weeks of culture. Bars represent the means ± SE. Bars denoted by the same letter within response variables are not significantly different (*p*= 0.05) using Duncan's multiple range tests.

MS basal medium exhibited a high efficiency for shoot regeneration and multiplication and WPM gave satisfactory results while B_5 medium exhibited comparatively low regeneration. The improved growth of cells and tissues on MS medium is undoubtly due to increased ammonium and nitrate concentration than WPM and B5 medium. Even, the concentration of potassium ion, inorganic nutrient and vitamins is higher in MS medium and therefore, it is widely used for tissue culture studies in many plant species. The findings are in agreement with earlier reports on many woody tree species including *Lagerstromia parviflora*, *Populus alba* × *P. berolinensis* and *Pterocarpus marsupium* (Tiwari et al. 2002; Wang et al. 2008; Husain et al. 2008), where MS medium proved best for highest shoot induction and multiplication.

Effect of pH value on shoot regeneration

The pH of the culture medium is an important factor for promoting shoots *in vitro*. In the absence of pH regulation, the ionization of acidic and basic groups causes considerable changes in structure that affect their function at the cellular level (Sakano 1990). The pH of tissue culture media decreases by uptake of NH_4^+ and increases by uptake of NO_3^- (Schmitz and Lorz 1990). A change of medium pH may have various effects that may influence performance and development of explants (George et al. 2008). In this study, a better performance in all parameters on shoot development was found at pH 5.8 among all pH levels

tested (5, 5.4, 5.8, 6.2, and 6.6) on MS medium containing 7.5 µM BA and 0.5 µM NAA (Fig. 4). Similar response have been observed by Gautam et al. (1993) where the proliferation of shoots in *Azadirachta indica* was significantly increased when the pH of the culture medium was adjusted to 5.8 before autoclaving. Meanwhile, Nair and Seeni (2003) also found the best shoot multiplication at pH 5.8 level in *Calophyllum apetalum*. Karim et al. (2007) reported in-*Aralia elata* that lower and higher pH levels than 5.8 showed a low performance for the induction and elongation of shoots from leaf derived calli. The main reason for such results seems to be the semisolid status of the medium at pH 5.8 as in semisolid media, the availability of many compounds is pH dependent, pH play important role in enhancing the activities of growth regulators and enzymes that affect the function of cells as well as whole plants (Scholten and Pierik 1998; Van Winkle et al. 2003) and a lower pH resulted in unsatisfactory solidification of agar.

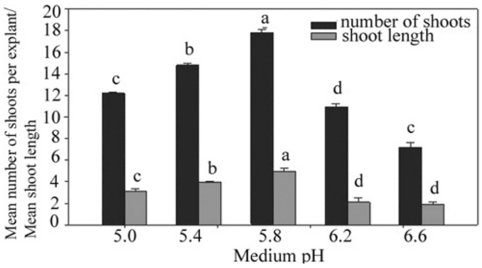

Fig. 4 Effect of medium pH on shoot regeneration from root explants of *A. lebbeck* supplemented with BA (7.5 µM) + NAA (0.5 µM) after eight weeks of culture. Bars represent the means ± SE. Bars denoted by the same letter within response variables are not significantly different (p= 0.05) using Duncan's multiple range tests.

In vitro rooting of shoots

The regenerated microshoots (4–5 cm) were excised and transferred to full and half strength MS basal medium for rooting. Root formation was noticed from the base of shoots after four weeks of culture. Half strength growth regulator free MS medium was found superior to full strength. For better rhizogenesis, half strength MS medium supplemented with different concentrations of auxins IBA and NAA was tried. The best rooting was achieved in half strength MS medium fortified with 2.0 µM IBA where fairly good root number (5.20±0.83) and root length per shoot (4.40±0.67 cm) were obtained (Table 3) (Fig. 1d). IBA has been reported to have stimulatory effect on root induction in many tree species including *Morus indica* (Chand et al. 1995), *Murraya koenigii* (Bhuyan et al. 1997), *Sterculia urens* (Hussain et al. 2008), *Balanites aegyptiaca* (Anis et al. 2009).

Acclimatization

The rooted plantlets were excised from proliferated cultures and washed carefully to remove adherent agar particles and planted to plastic pots containing sterile soilrite. The potted plants were covered with transparent polythene bags to ensure high humidity. After one month, surviving plants were transferred to pots containing normal garden soil and maintained in greenhouse (Fig. 1e). They were indistinguishable morphologically from normal-grown seedlings and survival rate was > 80%.

Table 3. Effect of MS strength and auxins concentration on rooting of *in vitro* raised microshoots of *A. lebbeck* after four weeks of culture

Treatments	% Response	Mean no. of roots/shoot	Root length
MS	8.66 ± 0.66^{f}	1.80 ± 0.58^{e}	1.54 ± 0.43^{e}
1/2MS	11.33 ± 1.20^{f}	2.60 ± 1.14^{de}	2.10 ± 0.33^{cde}
1/2MS + IBA (0.5 µM)	30.33 ± 7.63^{cde}	3.80 ± 0.83^{abcd}	2.80 ± 0.66^{bcde}
1/2MS + IBA (1.0 µM)	44.33 ± 2.33^{c}	3.60 ± 1.51^{bcd}	3.60 ± 0.50^{abc}
1/2MS + IBA (1.5 µM)	54.66 ± 2.40^{b}	4.40 ± 0.89^{ab}	3.74 ± 0.16^{ab}
1/2MS + IBA (2.0 µM)	66.00 ± 1.52^{a}	5.20 ± 0.83^{a}	4.40 ± 0.67^{a}
1/2MS + IBA (2.5 µM)	62.00 ± 0.57^{a}	4.20 ± 0.83^{abc}	3.00 ± 0.54^{abcde}
1/2MS + NAA (0.5 µM)	32.33 ± 1.45^{e}	2.80 ± 0.83^{cde}	2.00 ± 0.35^{dc}
1/2MS + NAA (1.0 µM)	36.33 ± 2.18^{de}	3.20 ± 0.83^{bcde}	2.10 ± 0.33^{cde}
1/2MS + NAA (1.5 µM)	41.33 ± 0.88^{cd}	3.60 ± 0.89^{bcd}	2.60 ± 0.50^{bcde}
1/2MS + NAA (2.0 µM)	54.66 ± 2.40^{b}	4.00 ± 1.22^{abcd}	3.40 ± 0.28^{abcd}
1/2MS + NAA (2.5 µM)	50.33 ± 0.33^{b}	3.60 ± 0.89^{bcd}	2.20 ± 0.48^{cde}

Conclusion

The protocol described in this study for regeneration of *A. lebbeck* using root explants of axenic seedlings is reproducible and improved method which could be useful for conservation and large scale planting of this economically and medicinally important tree legume.

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